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# Tumour necrosis factor-alpha (TNF-α), interleukin-10 (IL-10) and interferongamma (IFN-γ) in leprosy

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Leprosy is a chronic infectious disease characterized by a broad spectrum of clinical forms depending on the patient's immune response, in particular cell-mediated immune response. Cytokines can play a role in the cell-mediated immune response. Serum levels of Interferon-gamma ( IFN-γ ), Interleukin-10 (IL-10), Tumour Necrosis Factor (TNF-α), were measured by enzyme-linked immunosorbent assay (ELISA) in 45 leprosy patients with reaction and 30 age and sex matched healthy controls. Significantly higher serum levels of the cytokines were detected in leprosy patients compared to controls. Serum cytokine levels of paucibacillary (PB) patients have significantly higher level of IFN-γ and TNF-α than those of multibacillary (MB) patients. MB patients have significantly higher serum level of IL-10 than PB patients. Patients with type I reaction have high levels of IFN-γ and those of type II reaction have increased level of IL-10. In leprosy patients, IFN-γ and TNF-α are immunoprotective and IL-10 is immunosuppressive. Results indicate that type I reaction is a cell mediated immune response and type II reaction is essentially an immunocomplex disease.

## INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, which is an obligated` intracellular pathogen [1]. It is characterized by a broad spectrum of clinical forms depending on the host's immune response, in particular cell-mediated immune (CMI) response, which is responsible for the defense against intracellular pathogen [2]. Patients exhibit a strong CMI response to *M. leprae* at the tuberculoid pole and it declines toward the lepromatous pole where patients exhibit a defective CMI response to *M. leprae* [3].

Cytokines act as molecular signals for communication between cells of immune system and play a role in CMI response of leprosy patients. Cytokine dysregulation is responsible for selective defect in the CMI response to *M. leprae* in lepromatous patients [4, 5]. Skin lesions of MB patients showed an increased production of the suppressive T-helper 2 ($TH_2$) cytokines (IL-4, IL-5 and IL-10) [6], a decreased production of the protective T-helper 1($TH_1$) cytokines (IL-2 and IFN- γ) and macrophage cytokines (TNF-α) [4, 6].

Acute episodes "reaction" may occur during the chronic course of leprosy. The reactional states are classified as either type I (reversal reaction) or type II (erythema nodosum leprosum, ENL) [7]. The former is attributed to a rapid change in the CMI response [8] and the latter to immune complex pathogenesis [9]. However, CMI response has been shown to play a role in its pathogenesis [10]. Cytokines, especially the proinflammatory cytokines (TNF-α), may be involved in reactional states of leprosy [11, 12].
*In vivo* and *in vitro* studies are used to delineate the immunologic aspects of

leprosy and its reactional states. *In vivo* studies are better tools to detect what actually occur in patients and one of them is the measurement of serum cytokines produced by cells of the immune system in response to *M. leprae*. It is a simple and less expensive method compared to other *in vivo* approaches e.g. immunohistochemical and polymerase chain reaction (PCR) studies. The aim of the study is to delineate the serum cytokine levels of leprosy patients with reaction.

## MATERIALS AND METHODS

Forty-five leprosy patients (25 PB and 20 MB) classified according to WHO criteria [14] from the Central Special Skin Clinic (CSSC), Yangon General Hospital and 30 age and sex matched healthy controls from National Blood Bank were studied. Of these cases, 36 patients have reactions (20 type I and 16 type II).

Detailed history taking, medical and dermatological examination, slit-skin smear for bacteriologic and morphologic indices and serum samples were collected from all patients. Serum samples were aliquoted into 0.5 ml volume and stored at –80ºC until tested. TNF-α, IL-10 and IFN-γ cytokine levels were measured by enzyme-link immunosorbent assay (ELISA) (TECHNE Corporation, Minneapolis, U.S.A) with the lower detection levels of 10pg/ml, 5pg/ml and 100pg/ml, respectively. The data were analyzed using SPSS software. Student's *t*-test was used to compare the patients and the control group.

## RESULTS

All patients have significantly high serum cytokine levels compared to controls MB patients have high IL-10 ($p<0.001$) and low IFN-γ and TNF-α ($p<0.001$) compared to PB patients (Table 1). Patients with type I reaction have high IFN-γ ($p<0.001$) (Table 2) and those of type II reaction high IFN-γ and IL-10 ($p<0.05$) (Table 3).

Table 1. Serum cytokine levels of leprosy patients and control

| (pg/ml) | Control | PB | MB | Whole group |
|---|---|---|---|---|
| IFN-γ | 148.00 ± 31.81 | 1015.63 ± 227.12* | 460.67 ± 254.57* | 707.31 ± 367.87* |
| IL-10 | 32.05 ± 7.65 | 55.58 ± 17.65* | 92.45 ± 23.72* | 78.87 ± 30.82* |
| TNF-α | 13.15 ± 4.02 | 76.66 ± 52.16* | 31.35 ± 20.72* | 50.12 ± 43.72* |

*p <0.001

PB = paucibacillary MB=multibacillary

Table 2. Serum cytokine levels of PB with and without Type I reaction

| (pg/ml) | PB | PB with Type I reaction | *P* |
|---|---|---|---|
| IFN-γ | 833.7 ± 279.46 | 1260 ± 191.4 | *** |
| IL-10 | 70.46 ± 23.16 | 66.67 ± 9.82 | NS |
| TNF-α | 54.67 ± 46.25 | 34.86 ± 24.51 | NS |

*** $p$ <0.001

PB = paucibacillary NS = not significant

Table 3. Serum cytokine levels MB with and without Type II reaction

| (pg/ml) | MB | MB with Type II reaction | *P* |
|---|---|---|---|
| IFN-γ | 346.00 ± 180.0 | 562.50 ± 173.68 | * |
| IL-10 | 94.5 ± 4.94 | 111.0 ± 21.07 | * |
| TNF-α | 32.10 ± 20.67 | 35.00 ± 14.14 | NS |

*$p$ <0.05

MB = multibacillary NS = not significant

## DISCUSSION

Cell-mediated immunity is responsible for defense against intracellular pathogens, such as *M. leprae*. $TH_1$ cells are activated at the tuberculoid pole resulting in strong cell-mediated immunity whereas activated $TH_2$ cells in turn inhibit $TH_1$ cells at the lepromatous pole leading to defective cell-mediated immunity [18].

All patients have significantly higher serum cytokine levels compared to controls indicating stimulation of cells of the immune system by *M. leprae* antigens even in MB patients with known T-cell unresponsiveness to *M. leprae* [19].

PB patients have significantly higher serum IFN-γ and TNF-α levels compared to MB patients indicating activation of $TH_1$ cells which are the major source of IFN-γ [20]. T-cells from PB patients stimulated with *M. leprae* produced high levels of IFN-γ compared to that of MB patients [21, 22]. In addition, it has been shown that skin lesion of PB patients showed significantly high IFN-γ production compared to MB patients [4-6]. IFN-γ plays a central role in protection against intracellular bacteria [23]. The protective value of IFN-γ in leprosy was evident from the reduction of viable *M. leprae* organisms at the site of intradermal injection of IFN-γ [24, 25].

When the two types of leprosy reaction were compared, type I patients had significantly elevated levels of IFN-γ while type II patients had significantly elevated levels of IL-10. These findings could explain the difference in the pathogenesis of the two types. Type I reaction is a CMI reaction and the role of IFN-γ in cell-mediated immunity is well known [26]. Type II reaction is essentially an immune complex disease and the role of IL-10 in augmenting humoral immune responses is well documented [27].

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